Folic acid fortification of foods

Estimated intake of folate from different fortification scenarios
in
Norwegian population groups

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Summary

Clinical deficiency of micronutrients is uncommon in the developed world, but interest has increasingly focused on non-clinical deficiencies, or suboptimal status of micronutrients and the effect such deficiencies may have on risk of chronic disease. The focus on folate has greatly evolved over the past two decades from the prevention of anaemia to the prevention of neural tube defects (NTD). Folate also appears to have a lot of other health benefits; accumulating evidence suggests that folate may play a role in the prevention of cardiovascular disease and cancer. This expanding role of folate in prevention has major public health implications

Public health policies have developed in two ways: some American and developing countries have chosen mandatory fortification of staple foods with folic acid (FA), while most western European countries so far have decided not to do so. In most Western European countries, voluntary fortification is nevertheless allowed, and some specific products are available.

In this thesis the main aim was to: "determine a level of mandatory fortification that maximised folic acid (FA) intake for women of fertile age, to assist in achieving their recommended intake of 400 μg FA/day, whilst preventing a significant proportion of people in the target and non-target groups to exceed the upper safe levels of intake. In considering mandatory fortification of food with FA, flour and bread were selected as the food vehicles. Breakfast cereals, juice, nectar, yoghurt and milk were selected as possible products for voluntary fortification. The effect of various fortification and supplementation scenarios have been studied for children 4, 9 and 13 years old, adult men, women of child-bearing age and pregnant women from. *Norkost II (1997), Ungkost II (2000-2001)* and the *Norwegian Mother and Child Cohort Study*.

According to the results it is impossible with mandatory fortification of food with FA in Norway at a level that ensures the majority of women in the target group will consume 400 μg folate/day, without other population groups exceeding upper safe

level for FA. If mandatory fortification of flour with FA would be 140 µg FA/100 g flour, 41% of the fertile women and 29% of the pregnant women would reach the recommended intake. In the same scenario almost 20% of the children aged 4 and 9 years would exceed upper level. The scenario where least persons got an intake over upper level was when bread was fortified with 100 µg FA/100 g. In this scenario 19% of the children aged 4 years and 15% aged 9 years reaches upper level, whereas over 50% of the fertile and pregnant women did not reach the recommended intake.

With intake of voluntary fortified foods 83% of the fertile women and pregnant women would reach the recommendations, if taking FA supplement with 200 µg/day in addition to dietary folate. In the fortification scenarios for those taking supplements with 200 µg FA in addition to the five voluntary products and dietary folate show that 99% of children aged 4 years and 75% aged 13 years received total folate over UL. The fact that some of the supplements designed for children contain 200 µg FA should be questioned, especial if Norway goes for mandatory fortification but also with regards to voluntary fortification. It might be a good decision to limit the content of FA in supplements designed for children.

Data on the long term effects of increased folate intake are limited. The effects of long term exposure to high concentrations of FA are unknown, but antimetabolite effects are theoretically possible. We need to know more about the effects of chronic exposure to FA before instituting mandatory fortification. The strategy of universal fortification of staple foodstuffs with folic acid also presents the possibility of life-long exposure to unmetabolized FA. Therefore Norway should wait for new knowledge before starting mandatory fortification

Innhold

Abbreviations

5-MTHF	5-metyl tetrahydrofolate
AACR	American Association for Cancer Research
ALA	Acceptable level of addition with nutrient per 100 kcal portion of food
CHD	Coronary Heart Disease
CI95	High micronutrient intakes in Europe at the 95th percentile intake for each nutrient, used in fortification models
COMA	(US) Committee on Medical Aspects of food and Nutrition Policy
DF	Dietary folate
DFE	Dietary Folate Equivalents (1 μg folic acid = 1,7 μg DEF)
EAR	Estimated Average Requirement
EI 95	The 95th percentile energy intake
EUROCAT	European Surveillance of Congenital Anomalies
EVM	Expert Group on Vitamins and Minerals
FA	Folic Acid
FAO	Food and Agriculture Organization of the United Nations
FDA	(US) Food and Drug Administration
FFQ	Food Frequency Questionnaire
GLs	Guidelines Levels

Hcy	Homocystein
HOPE	Heart Outcomes Prevention Evaluation
HPCL	High Performance Liquid Chromatography
IE 96	Food Composition Date Base used in KBS
KBS	Software system for calculation of food, energy and nutrient intake
MA	Maximum amount
MoBa	The Norwegian Mother and Child cohort study
MRC	British Medical Research Council
MTHFR	Metylenetetrahydrofolate
NAHNES	National Health and Nutrition Examination Survey
NNR	Nordic Nutrition Recommendations
NORVIT	Norwegian Vitamin Trials
NTD	Neural Tube Defect
PBA	Protein Building Assay
PFFn	The factor of food in the market which is available for fortification
PGA	Pteroyl Glutamic Acid
RBC	Red blood cells
RI	Recommended intake
SAM	s-adenosylmethionine
SBU	Swedish Council on Technology Assessment in Health Care

SCF	Scientific Committee on Food
SI	Supplement Intake
SLF	Norwegian Agricultural Authority
SLV	Swedish National Food Administration
SPSS	Statistical Package for Social Sciences
TGLs	Temporary Guidance Levels
Thtcy	Total Homocystein
UL	Upper level
WHO	World Health Organisation

1. Bakground

Folate plays a central part in metabolism and in the maintenance of tissue function. An adequate intake therefore is necessary, but provision of excess supplements to people who do not need them may be harmful.

1.1 Folate

Folate was isolated in 1941 from spinach and named after the Latin word folium as means leaf. The initial reason for research on folate metabolism, was to find a cure for anaemia, it was soon recognized that the administration of folate enhanced the growth of existing tumours and that folate metabolism may be a promising target for anticancer drug design (1).

1.1.1 Function

Natural reduced folates are important vitamins that are essential for the eukaryotic cell and cannot be synthesized in the human body. Many different plants and bacteria serve as folate resources. Folate is a generic term for a naturally occurring family of B-group vitamins comprising an aromatic pteridine ring linked to p-aminobenzoic acid and a glutamate residue. Folic acid (FA), the fully oxidized monoglutamyl form, pteroyl monoglutamic acid (PGA) is the synthetic form used commercially in supplements and in fortified foods.

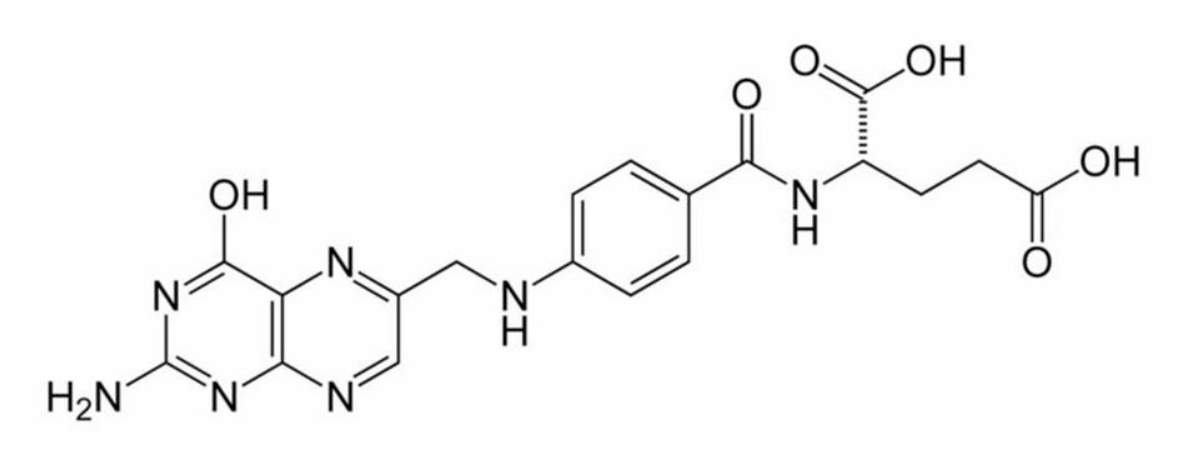


Fig.1 Folic Acid

As one-carbon donors and co-factors they play a role in a variety of biosynthetic reactions. As an essential cofactor for the de novo biosynthesis of purines and thymidylate, folate plays an important role in DNA synthesis, stability and integrity, and repair. Folates also regulate gene expression by providing methyl donors for DNA methylation (2), and in the remethylation of homocysteine (Hcy) to methionine, which is the precursor of S-adenosylmethionine (SAM), the primary methyl group donor for most biological methylations, including that of DNA (3).

Food folates must be hydrolysed by pancreatic and brush border folate conjugate to monoglutamates prior to absorption in the upper part of the small intestine (4), and then metabolized upon absorption, in the gut mucosa and liver, to 5-methyl tetrahydrofolate (5-MTHF), which is usually the only form found in plasma. In plasma the amino acid homocysteine concentration is a sensitive marker of folate and vitamin B12 status (5). Methylenetetrahydrofolate reductase (MTHFR) is a key regulatory enzyme in the metabolism of folate. The common C677T variant in the MTHFR gene results in a reduced activity of this enzyme, thereby increasing the availability of folate for the production of thymidylate and purine for DNA synthesis and repair (6).

Erythrocyte folate is considered to be the best indicator of long-term status since the lifespan of the erythrocyte is 120 days, and folate is retained in the erythrocyte for the duration of its life. Folate is incorporated into the developing erythroblast during

erythropoiesis in the marrow and less than 1% of circulating erythrocytes are replaced daily (4). Unlike serum folate, erythrocyte folate is not affected by recent or transient changes in intake, which may reflect actual intake (7). Macrocytic anaemia is one of the clinical manifestations of folate deficiency.

1.1.2 Sources

Folates are found in a wide variety of foods. Rich sources are liver, yeast extract, and green leafy vegetables such as Spinach, Kale, and Brussels sprouts (8). But cereal products, vegetables, fruits and potatoes appear to be the most significant sources of folate in the Norwegian diet. The Norwegian survey Norkost (n=2672) demonstrated that cereals and cereal products are the predominant sources of folate in the Norwegian population, providing approximately 27% of the daily intake of folate for women and 32% for men.

1.1.3 Bioavailability

Food folate bioavailability and the factors that affect it are poorly understood (9). A number of methodological approaches have been used to address food folate bioavailability, but there are some major limitations that may confound the interpretation of studies using these various approaches. Chronic bioavailability studies explore, usually in parallel design, the effect of long-term, typically 4–24 weeks, interventions with comparable doses of FA and reduced folates on markers of folate status. According to McNulty this type of studies with food folates are likely to give the most meaningful results in future research, but only if they are based on robust and tightly controlled protocols to ensure the delivery of food folate and FA at equivalent doses throughout the intervention period (9). Little is also known about the extent to which variation in the bioavailability of folate in foods would affect human nutritional status and health, but such associations undoubtedly exist, a variety of factors can contribute to incomplete bioavailability of naturally occurring folates from foods (10). Example of that is: folate status, alcohol (a well-known folate

antagonist), cigarette smoking (which depletes systemic and intracellular folate), and the supply of other methyl group donors (e.g., vitamins B6 and B12 and methionine) involved in one-carbon transfer (11).

Folic acid in fortified food has been found to be highly bio available (12). Whereas bioavailability of folate from diet is set to 50% (13). That is built on estimate of an old study by Sauberlich et al. from 1987, with a small number of subjects, and not all food sources of folate exhibit poor bioavailability. It has been suggested that an assortment of fruit and vegetables will provide dietary folate with 60–90% bioavailability relative to folic acid (14). Other evidence has shown that FA added to food has about 85% of the bioavailability of free FA (15).

Differentiation between folate forms is important since the individual folate derivatives show different stability and bioavailability. Many aspects of current folate requirements are being expressed in terms of dietary folate equivalents, in which an adjustment is employed to account for differences in mean bioavailability of natural and added forms of folate (10). It is nowadays common to use “Dietary Folate Equivalents” (DFE), which account for differences in the absorption of naturally occurring food folate and the more bioavailable synthetic folic acid. DFE may be expressed in different ways, depending on the type of conversion needed.

1 µg DFE = 1 µg Food folate = 0,6 µg Folic acid ,if it is added to food, and 0,5 µg if it is taken without food (16).

1 µg Folic acid = 1,7 µg DFE

1.1.4 Recommendation

Folate exists in many different chemical forms in foods and differences in its stability have led to difficulties in characterising the vitamin and establishing accurate data on food folate content. The most frequently used folate quantification methods are microbiological assays, protein-binding assays (PBA) and high performance liquid chromatography (HPLC). However, there is today no method with a status as

reference method for the measurement of natural folate in foods since all methods are complicated by difficulties in sample preparation, including extraction, deconjugation and purification (17).

In the Nordic Nutrition Recommendation (NNR) 1996, the recommended intake of folate was increased from 200 to 300 μg /day in order to provide a greater margin of safety and allow possibilities to increase store, especially in fertile women. Today the recommended daily intake of dietary folate in Norway is 300 μg for adults but 400 μg for fertile women (13). In NNR 1996, an intake of 400 μg/day was recommended from the beginning of the pregnancy. A daily intake of 400 is now recommended for all women capable of becoming pregnant. Pregnant and lactating women are now recommended a daily intake of 500 μg (13). Both FAO/WHO and the US authorities recommend all adults a daily intake of 400 μg, pregnant women 600 μg and lactating women 500 μg.

Table 1. NNR recommended daily intake of Folate

Recommended Folate intake	**μg/day**
Children 2-5 years	80
Children 6-9 years	130
Children 10-13years	200
Adults	300
Women of fertile age	400
Pregnant and lactating women	500

There is no evidence for risk associated with high intake of folates from natural sources. The safety of chronic very high intakes of folic acid is on the other hand largely unknown. The Institute of Medicine recommends a tolerable upper intake level for folic acid from supplements or fortified foods of 1000 μg/day for adults. These upper intake levels were developed primarily to avoid masking the anaemia and missing the neuropathy of vitamin B12 deficiency because very few data were available on other possible adverse effects of chronic high intakes. The clinical significance of the upper intake levels is not well established and they were instituted as a first attempt to raise awareness that not all levels may be safe. The European

Union Scientific Committee on Foods (2000) defines the upper level (UL) as: "the maximum level of total chronic daily intake of a nutrient (from all sources, including foods, water, nutrient supplements and medicines) judged to be unlikely to pose a risk of adverse health effects to almost all individuals in the general population." Individuals at greatest risk of exceeding the UL are those who consume large amounts of food, choose proportionally more fortified foods or take dietary supplements. The proportion of individuals at risk of excessive intakes can be estimated by comparing the 97.5 percentile intakes against the UL.

Oral folic acid, in excess of about 260 μg, can lead to the appearance of unmetabolised FA in the systemic circulation (18). The long term biological effects of this exposure are unknown (19). Although small doses of oral folic acid are efficiently metabolized to 5-MTHF before entering the portal blood, intakes > 200 μg appear to overload this metabolic capacity, leading to the appearance of unmetabolised FA in plasma.

A follow up placebo controlled double-blind randomized trial addressed the question of what level of FA per day would be required to increase a woman's red cell folate concentration to a protective level (20). That study demonstrated that 200 μg of FA per day might reduce neural tube defects by 39–41% and would be safer for the general population than 400 μg/day, which would be very effective but could result in unnecessarily high exposure for many people. 100 μg FA/day was predicted to result in a reduction of 22–26% in NTD over time (20).

Table 2. NNR: upper intake levels of Folic Acid μg per day

Upper intke levels of Folic Acid	**μg/day**
Children 1-3years	**200**
Children 4-6years	**300**
Children 7-10 years	**400**
Adolecent 11-14 years	**600**
Adolecent 15-17 years	**80**
Adults	**1000**

1.2 Intake in Norway

There are several problems involved in determining and interpreting folate intake. The quantity of folate in food must be known in order to interpret the relationships among food selection, folate intake, folate bioavailability and nutritional status.

1.2.1 Food folate

The average intake in the Nordic countries is estimated to 240-340 µg/10MJ (13). According to Norkost II a nation-wide survey of a representative random sample of the adult Norwegian population (n=2672) in 1997, the average intake of food folate was 309 µg for men, and 250 µg for women(21). In Ungkost, 2000-2001, the average intake of food folate was 196 µg for children aged 9 years (n=810) and 205 µg for those aged 13 (n=1005) (22). Data from 19711 women in the Norwegian Mother and Child Cohort Study (MoBa), in 2000-2001, found food folate intake of 272 µg for pregnant women.

According to NNR-04 a balanced diet following general dietary advice and recommended energy distribution will contain approximately 400 µg/10 MJ.

1.2.2 Supplement

In Norway, official guidelines from 1998 state that all women who may become pregnant should take a daily FA supplement of 400 µg from one month before pregnancy throughout the first 2–3 months of pregnancy, to reduce the risk of NTD.

According to Norkost in 1997 only 49% of the women and 29% of the men used a supplement containing FA. In 2001 Braekke and Staff evaluated the use of FA supplementation in approximately 1500 pregnant women in Oslo. Folic acid was found to have been used by 58% at any time during their pregnancy at the recommended dose of 400 µg folic acid (73% of the non-immigrant women and 19% of the non-western immigrant population). The majority of women did not take folic acid periconceptionally as recommended by health authorities. Only 2.3% of the

immigrant women vs. 21.8% of the non-immigrant women had used FA in the recommended way (23).

Data from 22 500 women in the MoBa with deliveries recorded in 2000–2003 have been analyzed. Of the women in the study had 72% had used supplements containing FA at some point before or during pregnancy but that only 10% had taken FA supplements regularly in the recommended way. They also saw that maternal education and marital status were strong predictors of using supplements (24).

1.3 Health benefits

The evidence that folate deficiency plays an important part in the pathogenesis of neural tube defect (NTD) is now beyond doubt. But folate don't only reduce NTD, it also has a lot of other health benefits.

1.3.1 Anomalies of the central nervous system

The original suggestion that folate deficiency, apart from causing megaloblastic anaemia, might also play a part in producing NTD was made by Brian Hibbard (1964), who demonstrated an association between folate deficiency and NTD. This was supported by others, as Smithells et al.(25).

Neural tube defects are the largest group of anomalies of the central nervous system and are a major cause of morbidity and mortality in infants, worldwide. It has a multifactorial aetiology incorporating a combination of genetic and environmental factors, such as nutrition. Many NTD can be prevented by taking 400 μg of folic acid or probably even less during the periconceptional period (20). The well documented reduction in neural tube defect risk induced by FA has prompted widespread health advisories promoting daily supplementation among all women of childbearing age. It is now generally accepted that between 50 and 70% of affected births are preventable by maternal ingestion of FA before and during early pregnancy. The evidence for this

is based on a substantial number of international trials and case-control studies during the past 20 years (1;20;26-31).

According to the special report from EUROCAT in 2003, there are 36 congenital birth-defect registries in Europe, scattered among 17 countries. The number of pregnancies affected by NTDs each year has been estimated to be 4000 in Europe. There is a higher prevalence in women of low socioeconomic status (25;32) and there is a variation in rates as related to ethnic and racial background (26). There is also geographic variation across Europe in the prevalence of NTD, with the United Kingdom and Ireland having the highest rates for several decades (32), as it is worldwide (26). The prevalence of NTDs has declined in many countries, in part because of prenatal diagnosis and selective termination of affected pregnancies, when allowed by the country. In Norway NTD are affecting approximately 70 pregnancies every year (33). According to data from Medical Birth Registry of Norway there hasn't been any reduction in the prevalence of NTD attributable to the recommendation to take folate (33).

The research first suggesting an association between NTDs and maternal deficiency or defective metabolism of folates was published in 1965. Epidemiologic studies that followed supported the hypothesis that FA supplementation reduced the occurrence of NTDs. The first conclusive randomized controlled trial, British Medical Research Council study (MRC), showed that women who had already had a pregnancy affected by an NTD could often prevent more than two thirds of recurrent cases by taking 4000 μg of FA daily (31).

Folic acid supplementation appears to correct a disturbance in folate metabolism rather than a shortage of dietary folate. While it is accepted that maternal folate deficiency is not the underlying cause of NTDs, lower plasma and red cell folate concentrations have been noted in mothers carrying affected foetuses and the risk of having an affected child was shown to be inversely proportional to maternal early pregnancy red cell folate concentration (30).

Reports have also suggested that multivitamin supplementation, with FA, reduces the risks of other specific congenital malformations. Several lines of evidence support an association between maternal use of a vitamin supplement with FA in early pregnancy and a reduced risk for offspring with orofacial clefts (34;35). They don't only show reductions in the occurrence of clefts in infants whose mothers took vitamin supplements containing FA. They have also shown that mothers' use of folic acid antagonist medications has been associated with an increased risk of delivering offspring with oral clefts (36). It is not clear if the same doses (400 μg) that reduce NTD will reduce the risk for oral clefts. In fact, as the use of fortified cereal and vitamin supplements has increased in Dublin, birth prevalence of NTDs has decreased dramatically while the birth prevalence of oral clefts has barely changed. A recent Norwegian report, based case-control study, shows that supplementation with FA in the periconceptional period reduces the risk of cleft lip, with or without cleft palate, in newborns (37). They didn't see any decreasing in cleft plate alone. It is possible that the dose of folic acid needed to prevent cleft palate is higher than the dose needed to prevent NTDs (35).

There is also increasing evidence that multivitamins with folic acid may reduce the risk of other congenital malformations such as cardiovascular defects (38;39), urinary tract defects (34;38), and limb-reduction defects (39;40). Exactly which nutrients should be taken is not clear, and it seems that multivitamin supplements containing 400 μg of FA may offer greater benefit than FA supplements alone (34).

1.3.2 Coronary Heart Disease

The association of homocysteine (Hcy) with Coronary Heart Disease (CHD) has generated much interest. Observational studies have consistently demonstrated that higher plasma Hcy level is associated with an increased CHD risk (41). Numerous studies of folate, Hcy and CHD have associated an increased intake of folate, and a reduced concentration of Hcy, with a reduced risk of CHD (42;43), and this association has been confirmed by meta-analysis (44;45). Randomized controlled

trials have shown that a moderate increase in folate consumption can substantially decrease Hcy levels (46).

Nevertheless, it is unclear whether a long treatment will benefit end points as myocardial and death. However, several intervention studies with FA supplementation are in progress to establish its effect on cardiovascular mortality and morbidity (47). Recently, two intervention trials involving large numbers of patients have been published. The Norwegian vitamin trial (NORVIT) looked at various combinations of B vitamins, or placebo in 3749 patients who had had an acute myocardial infarction. After a median of 40 months, there was a 27% reduction in Hcy in those treated with folate and B12, but no effect on myocardial events was seen. Contrary to expectations, there was a trend toward an increased rate of events among patients receiving B vitamins, in particular the combination of FA, vitamin B6, and vitamin B12 (48). In the Heart Outcomes Prevention Evaluation (HOPE)-2 study, 5522 patients with vascular disease or diabetes were studied over five years. They got a daily treatment either with the combination of 2.5 mg of FA, 50 mg of vitamin B6, and 1 mg of vitamin B12 or with placebo. Despite a reduction in Hcy, again vitamin treatment had no effect on myocardial events, but had a marginally significant effect on stroke (49).

Homocysteine–CHD associations may be confounded in a variety of ways. In some studies homocysteine levels are higher in smokers or people from less favourable socioeconomic backgrounds, and existing atherosclerosis could itself increase homocysteine levels, which would automatically lead to a positive association between Hcy and subsequent CHD (50;51).

1.3.3 Cancer

It is clear that all areas of research related to folate and disease have expanded, but interest in folate in relation to cancer has increased in particular (19). Epidemiologic studies over the past decade have suggested an inverse association of folate status with cancer. This has been assessed by dietary folate intake or by the measurement of

blood folate levels. The studies have observed the risk of cancer of: lungs, oropharynx, esophagus, stomach, colorectum, pancreas, cervix, ovary, prostate, brain and breast, and the risk of neuroblastoma and leukaemia (52;53). Among these, epidemiologic support for such a relationship is clearly most compelling for colorectal cancer (54). In a recent large, prospective population-based study of Swedish women and men, they report an inverse association between intake of folate from foods and the risk of pancreatic cancer (55). The association with dietary folate was independent of other known and potential risk factors for pancreatic cancer, including age, smoking, obesity, physical activity, and history of diabetes, and it persisted after controlling for consumption of fruits and vegetables, many of which are naturally high in folate. This findings are consistent with other larges studies (56;57).

Folate has consequently been of particular interest as a potential cancer protective agent because of the important roles it plays in nucleotide synthesis, as well as in the biological methylation of molecules such as DNA, RNA, proteins, and the phospholipids. Folate deficiency is associated with increased risk for several types of cancer, through disruption of DNA methylation, DNA synthesis and deficient DNA repair (58).

French et al have presented data showing that the incidence of neuroblastoma in Ontario, Canada, seems to have declined dramatically since the start of FA fortification of grains in Canada. Neuroblastoma is a cancer of early childhood and because it develops in utero, neuroblastoma also remains the most commonly diagnosed malignant tumour of infancy. The aggressive nature of this tumour makes this disease the most common cause of cancer-related death among children 1 to 4 years old (59). Another case–control study with Australian children demonstrated that folate supplementation during pregnancy reduced the risk of acute lymphoblastic leukemia, and that the protective effect of the MTHFR polymorphisms depended on adequate folate intake (60). Folate deficiency has been associated with uracil miss incorporation into DNA, leading to double strand DNA breaks during uracil excision

repair and increasing the risk of chromosomal aberrations that is presumably the onset of the leukemogenic process (61).

Furthermore, the value of folate supplementation during pregnancy was shown to prevent acute leukaemia in children. Krajinovic et al reported a reduced risk associated with a combination of genotypes. On stratification of patients into those born before and after January 1996, when the effects of recommended folate supplementation during pregnancy could be observed, Krajinovic et al observed that the protective effect was present only in children born before 1996. These results suggested that the associated risk with a combination of genotypes in the MTHFR gene was dependent on dietary folate status (62).

1.3.4 Neurological disorders

B vitamins are required for wellbeing and normal functioning of the brain. Adequate intake of folate and vitamins B12 and B6 are already known to be important for the prevention of haematological and irreversible neurological abnormalities (63). The relationship between B vitamin status and cognitive function has been of interest for many years. There is evidence of relationships between intake and status of folate and vitamin B12 with neurological, cognitive, and memory impairment (5), but results have so far been inconsistent (64).

Plasma total homocystein (tHcy) is a strong and independent risk factor for memory impairment, with a clear dose response relation (65), an elevated plasma level of Hcy is also a strong risk factor for Alzheimer's. A positive relation has been reported between various cognitive tests and intake or blood concentrations of B vitamins (63;66). Recently Ravaglia concluded that elevated plasma tHcy concentrations and low serum folate concentrations are independent predictors of the development of dementia and Alzheimer's (67). Campbell confirm that erythrocyte folate is related to dementia after controlling for age, gender, education, income, diabetes diagnosis, serum creatinine, and depressive symptoms. But it still needs more randomized

clinical trials with tHcy-reducing therapy to provide further evidence for a relation among tHcy, B vitamins, and cognition.

1.3.5 Other health benefits

As mentioned earlier chronic deficiency of folate in the diet can cause anaemia. Low concentrations of serum folate and vitamin B12 and elevated concentrations of plasma tHcy have also been associated with psychiatric disorders (68). Low serum folate has been associated with reduced bone mineral density in women, and responsible for the association between homocysteine and risk of osteoporotic fracture in elderly persons (69-71). Folic acid supplementation has also been shown to protect patients with rheumatoid arthritis from methotrexate related toxicity without jeopardizing the efficacy of methotrexate (72;73).

1.4 Health risk

Data on the long term effects of increased folate intake are limited. The effects of long term exposure to high concentrations of FA are unknown, but antimetabolite effects are theoretically possible (19). Neurological complications may be found in patients with vitamin B12 deficiency (74). This may not be the only possible risk of folate supplementation. For example, animal and some clinical studies have suggested that folate supplementation in higher doses may increase cancer risk and accelerate tumour progression (75). Moreover, an association between folate deficiency and spontaneous miscarriage has been identified (76).

1.4.1 Cobalamin deficiency

High intakes of FA may raise the possibility of masking cobalamin deficiency, especially in an elderly population (29;74). There is a metabolic interaction between vitamin B12 and FA. Folic acid supplementation can therefore correct anaemia associated with vitamin B12 deficiency but will not correct changes in the nervous

system that result from this deficiency. Permanent neurological damage may occur if vitamin B12 deficiency is left untreated. Before the role of vitamin B12 in causing megaloblastic anaemia was appreciated, vitamin B12 deficient patients were often treated with FA. In many cases the anaemia improved initially; however, the associated neurological damage progressed. This phenomenon has never been studied systematically for the obvious reason that patients would never knowingly be given the wrong treatment, particularly when the neurological damage associated with vitamin B12 deficiency is often irreversible (29).

Masking of vitamin B12 deficiency, i.e. deficiency without anaemia because of FA, does not appear to be increasing as a result of food fortification with folic acid, despite the current high exposures. Diagnosing vitamin B12 deficiency in the absence of anaemia can be very difficult because it depends on the recognition of sometimes subtle neurologic abnormalities. Therefore, it is encouraging that the proportion of patients with low vitamin B12 concentrations but without anaemia has not increased since fortification of grain with folic acid began in US (77).

It should be mentioned that the theoretical risk of masking anaemia caused by vitamin B12 deficiency has never been reported for a high intake of natural food folates and has been described among a small number of patients receiving high pharmacological doses of FA (ranging from 5 to 15 mg/day) only (78). In fact, our knowledge of how frequently masking occurs is limited, and no evidence of an increase in low vitamin B12 concentrations without anaemia after fortification has been observed in the United States, although the actual intake is considerably higher than expected and exceeds the limit of 1 mg/day for many people (77;79).

1.4.2 Cancer

While observational studies show lower cancer rates associated with increased folate intake, some case reports and animal experiments suggest opposite effects. Data from studies conducted in animal models of colorectal cancer have suggested that

exceptionally large doses of FA provided after microscopic neoplasia are established may promote rather than suppress carcinogenesis (80).

Charles et al. followed up a large trial of folate supplementation in pregnancy from the 1960s. They examined the association between folate status and death, and they also analyzed the effects of folate supplementation. Women taking high doses of folate throughout pregnancy may be more likely to die from breast cancer in later life than women taking no folate (81). In the study, increased risk was seen at 5 mg of folic acid daily, a high dosage recommended only in secondary prevention of NTDs. The report has been criticized for its small size, lack of biological plausibility, and for causing unfounded alarm in the general population (82).

In a recent prospective case-control study of 254 prostate cancer cases and 514 matched controls, it was made a similar observation (83). A statistically significant positive association was found between plasma folate and vitamin B12 levels and risk of prostate cancer.

The studies by Kim have shown that the dose and timing of folate intervention are critical in providing safe and effective chemoprevention; exceptionally high supplemental folate levels and folate intervention after microscopic neoplasia established in the colorectal mucosa promote rather than suppress colorectal carcinogenesis. Folate deficiency has an inhibitory effect, whereas folate supplementation has a promoting effect on the progression of established neoplasm. In contrast, folate deficiency in normal epithelial tissues appears to predispose them to neoplastic transformation, and modest levels of folate supplementation suppress, whereas supraphysiological doses enhance the development of tumours in normal tissues (75;80). And as he say, the implication of this issue is important because the optimal dose of folate supplementation must be determined for folate chemoprevention to be effective and safe in humans. Even if some similarities do exist, tumour development in chemical rodent models of colorectal cancer differs in several important histological, clinical, and molecular genetic aspects from that observed in humans.

Preliminary results from the first randomized controlled trial of FA for chemoprevention of colorectal polyps have been recently reported at AACR (American Association for Cancer Research) 96th annual meeting. More than 1000 participants with a recent history of colorectal adenomas were randomly assigned to 1 mg FA daily, with or without aspirin. Follow-up colonoscopies were scheduled 3 years after the initial endoscopy and supplementation continued until a second surveillance exam. In this trial, FA use did not prevent the recurrence of colorectal adenomas (84).

1.4.3 Miscarriage

Published reports on the relation between FA supplementation during pregnancy and risk for miscarriage have been inconsistent. Several studies have reported that folate deficiency and defects in folic acid and homocysteine metabolism are associated with an increased risk for pregnancy loss (85). By contrast, analyses of data of two randomized trials from the beginnings of 1990th, the MRC study from United Kingdom and data from Hungarian, have been analyzed in 1997 by Hook and Czeizel. In the data from Hungarian they concluded that preconception use of a multivitamin containing 800 μg folic acid was associated with a significant 16% increase in miscarriage rates compared with women who received trace elements (86). In addition, data from the MRC study including all women assigned preconception FA treatment, 4000 μg, and reported a nonsignificant 15% increase in miscarriage rates (86).The hypothesis these authors mentioned was that folic acid may influence the viability of foetuses with malformations.

A large population-based cohort study with 23,806 births and 2155 miscarriages of first pregnancies in China found no evidence that daily intake of 400 μg FA influenced their risk for miscarriages (87). Neither did a case–control study on the relation between plasma folate levels and the risk of spontaneous abortion. In this study, low plasma folate levels were related to a higher risk of miscarriages (76). In 2000 Windham et al reported that data from a prospective study of women in

California, interviewed during their first trimester of pregnancy, supported the conclusion of Hook and Czeizel that preconception vitamin increased the occurrence of miscarriage. In this study, a nonsignificant 14% increase in the occurrence of miscarriage among women who took vitamins during the prenatal period was observed and attributed to preconceptional FA use although the supplements taken were a mixture of vitamins not FA alone (88). The hypothesis of Windham et al., for this phenomenon is that folic acid/multivitamin use prolongs pregnancies that may have miscarried very early until they are more likely to be recognized later in gestation. The somewhat higher proportion of very early miscarriages between the 4th and 8th gestational weeks in the trace element group supports this idea.

1.4.4 Twins

Swedish researchers have reported that folate supplementation in early pregnancy increases the risk of twin births (89;90). And at least three other studies have also suggested the possibility of an increase in the occurrence of multiple births (91-93). The two Swedish studies described the increased occurrence of dizygotic twins. Only 6953 women reported the use of FA in early pregnancy among 576,873 women who gave birth during that period. After exclusion of women who reported involuntary childlessness and women who used ovarian stimulation, and adjusting for year of birth, maternal age, parity and smoking, the odds ratio for dizygotic twinning was 1.71 (90).

In a large population-based prospective cohort study among young women in China with 242,015 births and 1496 multiple births, no increase of multiple births was found in women who had taken 400 μg FA supplements compared with those who did not (94). Although the available evidence is not conclusive, the large study from China which includes many young mothers and where the use of ovarian stimulation is not common did not show an increased risk of twinning, after periconceptional FA use (94). In this paper, it was suggested that the effect seen in the Swedish study was

due to confounding from maternal age and subfertility, in spite of the fact that adjustments for these factors had been made.

Berry et al. suggest that in the Swedish studies is wholly or largely a false result based on misclassification of in vitro fertilisation, itself a known risk factor for twinning (95;96). In assessing the credibility of the original Swedish reports it is useful to consider whether there is a plausible mechanism by which dietary supplementation might increase twinning rates. One explanation can be if folate and/or multivitamin supplementation reduces the rate of early twin loss it would lead to an increase in the number of twin deliveries without any effect on the rate of twin pregnancy (97).

1.4.5 Other health risks

There are clear benefits in receiving folate to lower homocysteine. There is also evidence that excess periconceptional folate can increase the prevalence of the C677T-MTHFR TT genotype, which is associated with higher homocysteine levels (98). No one knows yet if increasing dietary folate would influence antifolate cancer chemotherapy. Folic acid also interferes with antiepileptic drug metabolism in epileptic patients and may alter the control of epilepsy by anticonvulsant drugs (98). Experimental studies have confirmed that folates are highly convulsant if the blood/brain barrier mechanism is circumvented. Fortunately, the risk to patients is small because of the barrier mechanism, but the bigger the dose, the longer the duration and the greater the damage to the blood/brain barrier, the higher the risk (99).

The strategy of universal fortification of staple foodstuffs with folic acid also presents the possibility of life-long exposure to unmetabolized FA. Kelly et al. showed that an intake of folic acid in addition to that in the diet of 266 μg/day results in significant amounts of unmetabolized folic acid in the blood in adults. This suggests that not all FA supplied is available for the remethylation of Hcy to methionine. The

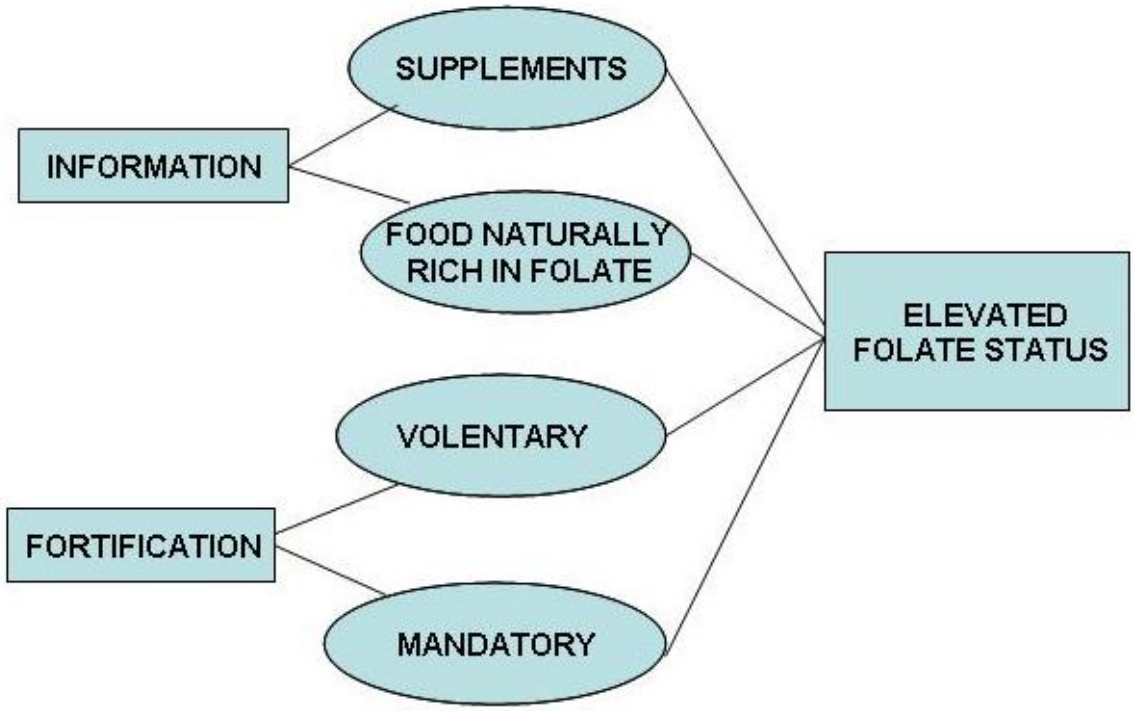
homocysteine-lowering response is thus not linear over the whole range, which implies an overestimation of the relative bioavailability for dietary folate (18).

1.5 Strategies

As mentioned earlier, there are several studies that indicated that increased intake of folate for women would reduce their risk of having babies with NTD. In Norway only few women reach the recommended 400 µg folate per day. There are several options for increasing the folate status of citizens:

- promotion of intake of foods naturally rich in folate
- recommendations on the use of folic acid supplements
- voluntary folic acid fortification of food
- mandatory folic acid fortification of foods

FIG 2: Several options for increasing the folate status of citizens



1.5.1 Promotion of intake of foods naturally rich in folate

To increase folate intake would require a considerable increase in consumption of foods rich in folate such as green leafy vegetables, legumes, and fruits. Even though this recommendation is included in most nutrition and health programs, it will take a long time to make such a change, and it may be difficult for all citizens to achieve a sufficient intake. But according to Brower et al. it is possible that the intake of folate-dense vegetables and citrus fruits significantly enhances the folate status and decreases tHcy concentrations in healthy volunteers. Her test diet compressed 350 g of vegetables, one piece of citrus fruit and 200 ml of citrus fruit juice in addition to the basal diet, thus achieving a total folate intake on 560 μg /day. This is higher than what can be expected to be eaten by the general population (14;100). An intervention study in healthy women, Cuskelly et al. found in contrast that dietary advice aiming at optimizing folate intake did not increase folate status, whereas supplementation with pills or fortified foods did. Folate as natural food folate is relatively ineffective at increasing folate status (101). Thus, improving dietary folate intake by health campaigns does not appear to be an efficient strategy to increase folate status of women of childbearing age sufficiently to prevent NTDs at a population level (101).

1.5.2 Recommendations on the use of folic acid supplements

In the EUROCAT report seven European countries, Norway included, had official health education initiatives to promote the use of FA supplements. In NNR 1996, an intake of 400 μg/day was recommended from the beginning of the pregnancy. In 1998, the *Norwegian Nutritional Council* recommended that women should take folate supplements prior to and during the first 2-3 months of pregnancy (33). This has been followed up with several information campaigns. In spite of this promoting the majority of women in all these countries in Europe were not taking folic acid supplements periconceptionally. This is reflected in the lack of a clear decline in the prevalence of NTD across Europe (32). It has also been shown that the highest use of

folic acid supplementation was found in countries that had introduced an official health education program to promote use of supplements (32).

According to several studies the use of FA supplementation by women is positively associated with age and socioeconomic status, and inversely with the number of pregnancies and with unplanned pregnancies (23;32;102). Almost twice as many women with a high educational level used FA in the advised way than those women with a lower educational level.

In a study of Botto et al. recommendations alone did not seem to influence trend in NTD up to six year after the confirmation of the effectiveness of folic acid in clinical trails (27). Botto and colleagues analyzed 13 birth registries from Europe and Israel and found no changes in the trend of incidence of NTDs between 1988 and 1998, even though the use of FA supplements had been promoted.

1.5.3 Fortification

McNulty et al. have demonstrated that a significant increase in dietary intake of food naturally rich in folate is less effective in increasing red cell folate status than is an equivalent amount of folic acid via fortified foods. This is thought to be because folic acid is more stable than natural folates and hence is more bio available (103).

Food fortification can provide relatively rapid solutions to address low micronutrient intakes at a population level, whilst maintaining traditional dietary patterns (104). However, concerns need to be addressed in relation to the hypothetical risk of over-consumption in individuals outside the at-risk groups or those who choose to eat high amounts of fortified foods (104). While there is evidence that food fortification can help address nutritional deficiency in some population groups, there is a risk of over nutrition in other population groups.

Fortification is defined by the Codex Alimentarius as the addition of one or more essential nutrients to a food, whether or not it is normally contained in the food, for the purpose of preventing or correcting a demonstrated deficiency of one or more

nutrients in the population or specific population groups (105). The fortification vehicle can be either a staple food, or a more-processed commercially-available food, and many have been tried.

Until now the Norwegian government has followed the principle that permissions for fortifications are only given for the benefit of health need, to supply the entire population or major parts of the population. Traditionally food fortification programs in Norway have been designed to:

- restore nutrients removed during food processing (iron in whey cheese)
- add nutrients in substitute foods (the addition of vitamins A and D to margarine, a replacement for butter)
- correct obvious deficiencies in populations (iodine fortification of salt, vitamin D in low fat milk)

Today the applications will be considered with focus on whether the fortification can make a health risk or not.

The recent WHO guidelines on fortification state that the goal of fortification should be to provide 97.5% of the individuals in a population with an intake that meets their Estimated Average Requirement (EAR) for specific micronutrients without exceeding their UL (106).

There are two ways to do it: Voluntary or Mandatory.

1.5.4 Voluntary folic acid fortification of food

Fortification of foods on a voluntary basis is allowed in many countries. In Norway it is not permitted to fortify food unless they have received permission from The Norwegian Food Safety Authority. In 1995 Australia's National Food Authority permitted the voluntary fortification of flour, bread, savoury biscuits, breakfast cereals, pasta, yeast extracts (a popular spread for bread in Australia), fruits and vegetable juices, and meal replacements with folate to 100 μg folate per serving (107). The voluntary fortification of foods with folic acid in Australia has been followed by a significant increase in serum folate and decrease in plasma

homocysteine in this community. The difference was greater in subjects who consumed at least one folate fortified food per week than subjects who did not use folate fortified foods (107). However, only a limited proportion of the permitted foods have been fortified under the voluntary folate fortification policy in Australia.

Breakfast cereals is the most frequently used FA fortified food in Europe, US and Australia. Voluntary fortified breakfast cereals contribute 15% of average daily folate intakes of British adults (108;109).

A public health decision in Hungary stimulated flour fortification on a voluntary basis, but it remains the only European country to take this action. Unfortunately, little is known about the proportion of bread and bread products that are actually made with fortified flour or about the effect on folate status or NTD rates in Hungary (110).

Only a few published studies are concerned with intake of fortified foods, and most are confined to single food groups or intake of specific nutrients. Godfrey et al. recently estimated the impact of fortified foods in France, Germany, Italy, Spain and the UK. They concluded that even for high level consumers of fortified foods, these consumers were unlikely to obtain more than 10% of their diet in a fortified form (111). But according to Rasmussen et al. food survey data for adults from the UK and France showed that for frequent consumers of fortified beverages (carbonated beverages, juices, fruit drinks and milk), these alone may contribute up to 17% of the total diet (112). A large range in the amount of fortified food consumed by children and some children can have a very high intake. In the German DONALD study from the years 1985–2001 the intake of fortified foods was highest in the youngest age groups and the intake of fortified foods in 2 to 3-year-olds contributed with 9.02±7.80 of the total energy intake.

1.5.5 Mandatory folic acid fortification of foods

Mandatory fortification has been started in about 40 countries throughout the world. Most of them are American but some are also African and Asian. Several European countries have advocated mandatory flour folic acid fortification over the last 6 years, but none has introduced it. In the United States and Canada, folic acid fortification of enriched grain products was fully implemented by 1998. Pfeiffer et al recently published the largest documentation of folate status since mandatory folic acid fortification of enriched cereal-grain products was initiated. These new data provide a comprehensive biochemical assessment of folate status in a representative sample of the American population in the National Health and Nutrition Examination Survey (NHANES) (68). The US population has shifted to significantly higher serum and RBC folate concentrations. There is observed increases across all subgroups of age, sex, or race-ethnicity. Adolescents and adults have experienced the biggest relative increase, children aged 5 year the smallest increase, and elderly persons an intermediate increase (68). The Centers for Disease Control and Prevention has reported that NTD rates have decreased 26% since fortification, but that additional effort is needed to achieve the national goal of a 50% reduction.

Recent reports indicate that the program is providing approximately twice as much folic acid as was originally estimated (79;113;114). This increase is higher than was expected from the 70–130 μg/d increase predicted by the US fortification program.

Since the implementation of folic acid food fortification in Canada, women's folate stores have increased dramatically in also in this country, 50% relative reduction in the risk of open NTD has been observed (115).

Fortification of wheat flour with folic acid has been shown to be effective in preventing NTD in Chile. In Chile, the FA fortification of wheat flour with 220 μg/100 g has been mandatory since January, 2000 (116). The mean intake of wheat flour as bread in Chile is very high, approximately 200 g/day. One year after fortification there is increases in serum folate and RBC folate of 3.8 and 2.4-fold,

respectively, in women of fertile age. Preliminary results show a reduction of 40% in the rates on NTD from the pre-fortification period (1999-2000) to post-fortification period (2001-June 2002) (116).

Even in countries with mandatory fortification policies, it is evident that some women are at more risk of a low folate status than others. A study in Georgia, USA, showed that low folate status was more likely for those not taking FA supplements and those less likely to eat breakfast cereal (fortified) regularly. It was also more likely for black women, smokers and those using a particular form of contraceptive injection (but not contraceptive pills in general) (117). Such women still need special targeting.

1.5.6 Models

Several models have been proposed to make the voluntary fortification safe (112;118-120). The most famous is the model by Flynn et al. They have published an empirical model, based on food intakes from several national dietary surveys across Europe, to calculate the level of each micronutrient that could be safely added to foods in Europe. A number of scenarios have been envisioned at considerably higher hypothetical proportions of foods fortified than those in current UK and Irish practice. Factors of the model include: micronutrient intakes at the 95th percentile level; proportion of fortified foods in the diets of consumers at the 95th percentile of food energy intakes; the proportion of foods in the diet to which micronutrients could in principle be added; the types of foods that might in practice be fortified. The model is based on the tolerable upper intake level (UL) established by the EC Scientific Committee on Food (SCF). The model is derived from adult intake data and UL established for adults. The results indicate that for all reasonably feasible scenarios all micronutrients could be safely added at nutritionally-relevant levels without exceeding the UL, with the exception of vitamin A.

Recently Rasmussen et al have published a model for the Danish Institute for Food and Veterinary Research. The Danish model is based on the model by Flynn et al. It employs three main elements, the ULs, the 95th percentile intake of micronutrients

from non-fortified foods (CI95) and supplements (SI), and the resulting maximum allowance (MA) for fortification. By using these factors, it is possible to estimate the level of each micronutrient that can be added to foods without any appreciable risk of adverse effects for any age group in the population, including individuals with high food intakes. The model is based on the following factors and mathematical formulae:

- UL: the tolerable upper intake level established by the SCF or other expert committees. Where no UL has been established, guidance levels (GLs) suggested by the UK Expert Group on Vitamins and Minerals (EVM) or temporary guidance levels (TGLs) suggested here are used instead;
- CI95: the current 95th percentile dietary intakes of micronutrients from non-fortified foods;
- EI95: the 95th percentile energy intake;
- SI: supplement intake; daily micronutrient intake from a normal vitamin/mineral supplement;
- MA: maximum allowance for intake of micronutrients from fortified foods: MA=UL – (CI95+SI);
- PFFn: the fraction of foods in the market which is available for fortification;
- ALA: Acceptable level of addition with each nutrient per 100 kcal portion of the food (112).

ALA = MA / EI95 * PFFn

The main difference between the two models is that the Danish model takes into account the regular use of a vitamin and mineral supplement and differentiates ULs for children of all ages. They also estimate that 50% of fortifiable foods will be fortified and, hence, that the MA can be distributed among only 25% of the energy intake.

Norwegian Scientific Committee for Food Safety have evaluated the Danish model for addition of vitamins and minerals to food, the conclusion is that it can bee a good instrument to assess fortification of food in Norway (121). The Authority wanted the prerequisite in the model that 25% of the energy is to be derived from fortified foods, to be reduced to 10 E%. When they used Norwegian data in the model they found

that the safe addition of FA to foods can be 20 μg/100 kcal without health risk to any consumer group.

2. Objectives

The aim of this study was to determine a level of mandatory fortification that maximised folic acid intake for the target group, women of child-bearing age, to assist in achieving their recommended intake of 400 μg of FA per day, whilst preventing a significant proportion of people in the target and non-target groups exceeding upper safe levels of intake. In considering mandatory fortification of food with folic acid, flour and bread were selected as the food vehicles.

The effect of various fortification and supplementation scenarios will be studied for children 4, 9 and 13 years old, adult men, women of child-bearing age and pregnant women.

The following dietary intake assessments were considered:

- Folate intake from food alone
- Folic acid intake from different mandatory fortification scenarios
- Folic acid intake from voluntary fortification of some foods
- Folic acid intake from voluntary fortification of some foods and supplement use
- Folic acid from mandatory and voluntary fortification and supplements
- Folates from food, , mandatory, voluntary fortification and supplements

3. Methods

3.1 Foods

Two potential food vehicles for folic acid mandatory fortification and six for voluntary fortification were selected.

3.1.1 Mandatory folic acid fortification

Wheat flour and bread were selected as potential food vehicles for folic acid fortification, since at least 50 g of these foods were consumed, by more than 90% of women of child-bearing age. Three fortification levels were used to examine the effect of mandatory fortifying of the selected food vehicles on folic acid intakes:

-Flour: 140, 190 and 240 μg/100 g flour

-Bread: 100 and 200 μg/100 g bread

The flour fortification levels was based on the concentration the US food and Drug Administration introduced in US (140 μg), in UK Committee on Medical Aspects of Food and Nutrition Policy (COMA) has proposed a concentration of 240 μg/100 g as a optimal level (122), and in Ireland 190 μg/100g is the level that Ireland is being considered.

The bread fortification levels were also based on different countries experiences.

Fortified bread began in Hungary in 1998, the daily intake of folic acid is estimated to about 100 μg in 100 g of bread (110).

Hertrampf et al. has assessed the effectiveness of the FA flour fortification program on bread in Chile. The mean folate concentration of the mean FA content of bread is 2020 μg/kg (116).

3.1.2 Voluntary folic acid fortification

Sweet and unsweetened breakfast cereals, juice, nectar, yoghurt and milk were selected as possible products for voluntary fortification. In the calculation of voluntary fortification 20 µg/100 kcal were used. This is based on the Norwegian Scientific Committee for Food Safety results then they evaluated "The Danish model for addition of vitamins and minerals to food".

Because the fortification level is per 100 kcal, breakfast cereals had to be calculated both as sweetened and unsweetened. For the same reason I had to calculate milk in four different ways. See appendix 1.

Table 3: Voluntary fortified 20 µg/100 kcal

Food	µg FA/100g
Breakfast cereals unsweetened	69
Breakfast cereal sweetened	77
Juice	9
Nectar	8
Yoghurt (3% fat)	16
Milk, whole milk	13
Milk, low fat 1,5%	9
Milk, low fat 0,7%	8
Milk, skimmed	7

3.1.3 Supplement

Folic acid intakes from dietary supplements (200 µg) were added to the individual folic acid intakes for three fortification scenario to yield total folic acid intakes.

3.2 Food intake data

The food data is from three large Norwegian dietary surveys, among children aged 4, 9 and 13 years, men aged 16-79, females aged 16-49 years and pregnant women.

3.2.1 Ungkost II

In 2000, a nationwide dietary survey using 4-days records with pre-coded food diary was conducted in representative samples of schoolchildren in the 4th and 8th grades. The pre-coded diary has been validated (123). In 2001 a nation-wide study was conducted among 4-year-old children using a similar design as among these 9- and 13-year-old students. The surveys were collaboration between the Norwegian Directorate for Health and Social Welfare, Department of Nutrition, Norwegian Food Control Authorities and the Institute for Nutrition Research, University of Oslo. The 4-day period consisted of three weekdays and one weekend day. Participants in the 8th grade recorded their diet themselves, while parents filled in the diary together with the 4-years old and students in the 4th grade. Along with the diary each participant received a photographic booklet which embodied 13 colour photograph series, each with four different portion sizes ranging from small to large. As 83% of the selected subjects participated, the data may be considered as representative for this age group in Norway.

3.2.2 Norkost II

The Norkost study was a nation-wide survey of a representative random sample of the adult Norwegian population and was undertaken by the National Nutrition Council in collaboration with the National Food Authority, Institute for Nutrition Research and the Norwegian Statistical Office. Approximately five thousand subjects were recruited and 2672 questionnaires were found adequately completed (participation rate 53%). A self-administered, quantitative food-frequency questionnaire (FFQ) was used for the data collection. It was designed to cover the whole diet including approximately 180 food items, grouped according to the Norwegian meal pattern. The selection of foods, portion sizes and frequencies was based on experience gained from earlier dietary surveys and the food frequency questionnaire has been validated (21;124)

3.2.3 The Norwegian Mother and Child Cohort Study

This study is a subproject in the Norwegian Mother and Child Cohort Study (MoBa) (125). In brief, MoBa is a pregnancy cohort that in the period 1999 to 2006 has included more than 75 000 pregnancies, and that aims to include 100 000 by the end of 2007. See appendix 2.

The sample used here includes 19.711 pregnancies using version 1 of the quality-assured data files made available for research in 2005. Here the pregnant women answered version 2 of the food frequency questionnaire, which has been validated (126). The data was collected from February 2002 until April 2003, and the women filled in how often they have been eating different food items since they became pregnant. The women answer the questionnaire in week 17-18 of the pregnancy. Reported intakes of foods and supplements are converted into daily intakes by FoodCalc (127) and the Norwegian food composition table (8).

3.2.4 Handling of data

From the "Ungkost" and "Norkost" surveys, data was computed for each person by using a food database and software system for calculation of food, energy and folate intake, developed at the Institute for Nutrition Research, University of Oslo, called KBS. The food database was based on the official food composition table, from 1995, (8). KBS is continuously supplemented with data on new food items.

KBS were used to find intake of bread, sweet and unsweet breakfast cereals, juice, nectar, yoghurt and milk with different fat content, for each person. In KBS it is also possible to find the intake of raw material, there I could find total intake of wheat flour from bread, cakes and flour containing dishes, for each person. I have received the corresponding data from the MoBa, except folate from each food product.

Data analyses were performed with the statistical software Statistical Package for Social Sciences (SPSS) 12.0 for Windows. The result from KBS and the MoBa, was saved as an Excel-file and imported to SPSS for further analyse.

The intake of wheat flour, bread and the selected foods for voluntary fortification (breakfast cereals, juice, nectar, yoghurt and milk) are presented in table 4 as mean intake and percentiles 5 and 95 per person per day.

Table 4 Intake of wheat flour, bread, breakfast cereals, juice, nectar, yoghurt, and milk g/day mean (p5 - p.95)

Product	Children 4 y n = 391	Children 9 y n = 810	Children 13 y n = 1005	Men 16-79 y n = 1291	Women 16-49 y n = 928	Women pregnant n = 19711
Wheat flour mean, g/d p5-p95, g/d	 66 27-113	 92 36-165	 96 30-193	 141 62-248	 98 43-167	 117 36-229
Bread mean, g/d p5-p95	 94 41-174	 115 43-217	 121 35-239	 212 90-390	 143 60-245	 222 61-406
Breakfast cereals mean, g/d p5-p95, g/d	 13 0-50	 17 0-86	 13 0-65	 14 0-67	 10 0-46	 11 0-55
Juice mean, g/d p5-p95, g/d	 26 0-131	 48 0-150	 58 0-267	 62 0-288	 68 0-300	 108 0-300
Nectar mean, g/d p5-p59, g/d	 18 0-122	 21 0-266	 18 0-133	 14 0-54	 18 0-108	 72 0-400
Yoghurt mean, g/d p5-p95, g/d	 58 0-163	 42 0-165	 37 0-175	 21 0-112	 32 0-126	 66 0-210
Milk mean, g/d p5-p95, g/d	 293 57-573	 345 35-735	 274 0-725	 466 10-1154	 316 4-801	 488 39-1278

y = years of age
p5-p95 = percentiles 5 to 95

The observations in the surveys have a distribution that is similar to a normal distribution, so the results are presented as mean. Some of the results are also given at percentile 5 and percentile 95.

4. Results

4.1 Folate intake from food

Women aged 16-49 was the group who had highest concentration of folate in their diet, the mean intake of dietary folate was 309 µg /10 MJ. The pregnant women had a mean intake at 275 µg /10 MJ which is similar to the men's mean at 288 µg/10 MJ (table 5).

As shown in table 5, the average pregnant woman had a higher folate intake (272 µg/day) compared with the average among fertile women (250 µg). Despite this it is still only 50% of the RI at 500 µg/day. Only 3% of the fertile women had an intake according to RI (400 µg). For men the average intake of diet folate was 309 µg, even if that is similar to the recommendations, 54% get an intake below RI (table 5). Almost all children aged 4 and 9 years, but only half of those aged 13 reached the RI. The average intake of diet folate was 153 µg for children aged 4 years, 196 µg for those aged 9 years and 220 µg for those aged 13.

Table 5: Intake of dietary folate (µg/day), energy (MJ/day) and dietary folate per 10MJ and the percentage of folate according to NNR recommendations.

	Children 4 y n = 391	Children 9 y n = 810	Children 13 y n = 1005	Men 16-79 y n = 1291	Women 16-49 y n = 928	Women pregnant n = 19711
Intake of diatery folate mean, µg/d p5-p95, µg/d	 153 89-239	 196 107-295	 220 99-408	 309 172-511	 250 133-397	 272 143-452
Intake of energy mean, MJ/d p5-p95, MJ/d	 6,2 4,3-8,4	 8,2 5,3-12	 8,8 4,6-14,1	 11,0 6,0-18,4	 8,3 4,7-13,1	 9,9 6,1-15,1
Diatery folate concentration mean, µg/10 MJ p5-p95, µg/10 MJ	 245 170-334	 241 167-335	 252 152-418	 280 210-391	 302 208-426	 275 182-394
Intake of dietary folate % ≥ RI	 98	 89	 45	 46	 5	 3

y = years of age
p5-p95 = percentiles 5 to 95
RI = Recommended Intake (see table 1 for recommended levels)

In table 5 the percentage of how many would meet or exceed the NNR recommendations are presented. When considering the possible adverse effects of high intakes of folate, it should be borne in mind that the NNR just has UL for intake of folic acid. No UL is set for food folate. Here is also the percentage given for the intake below NNR recommendations, there is not the same as lower level.

4.2 Different mandatory fortification scenarios

If mandatory fortification of flour with folic acid was introduced in Norway at the level decided by FDA in USA in 1998 (140 µg FA/100 g flour), the average adult woman below 50 years would receive 387 µg folate per day, i.e. they would not quite reach the recommended intake level of 400 µg folate per day (table 6). As shown in table 7, 41% would meet or exceed the recommended level, compared to 71 % if the higher level now proposed in England (240 µg) was implemented.

As shown in table 6, the average pregnant women would receive 436 µg per day if Norway should introduce mandatory fortification at 140 µg/100g flour, compared to 553 µg if the mandatory level would be 240 µg/100 g flour. Because the recommended level is 500 µg per day for the pregnant women, only 29% or 55% would reach the RI (table 7).

Table 6: Intake of FA in different mandatory fortification scenarios with and without dietary folat, µg per day, mean (p5 - p.95)

Fortification level	Children 4 y	Children 9 y	Children 13 y	Men 16-79 y	Women 16-49 y	Women pregnant
140 µg FA/100 g flour						
FA only						
mean, µg/d	92	129	150	198	137	164
p5-p95, µg/d	38-159	50-231	44-336	86-347	61-234	51-321
DF + FA						
mean, µg/d	245	324	354	507	387	436
p5-p95, µg/d	140-380	177-505	154-669	277-812	214-593	221-727
190 µg FA/100 g flour						
FA only						
mean, µg/d	125	175	198	269	186	223
p5-p95, µg/d	52-215	67-313	59-410	117-471	83-318	69-436
DF + FA						
mean, µg/d	278	370	402	577	436	495
p5-p95, µg/d	158-433	199-590	170-765	550-311	240-672	246-836
240 µg FA/100 g flour						
FA only						
mean, µg/d	158	221	246	339	235	282
p5-p95, µg/d	66-272	85-395	75-493	148-595	104-401	87-550
DF + FA						
mean, µg/d	311	416	450	648	483	553
p5-p95, µg/d	174-489	220-675	190-855	344-1077	261-746	267-946
100 µg FA/100 g bread						
FA only						
mean, µg/d	94	115	121	212	143	222
p5-p95, µg/d	41-174	43-217	35-239	90-390	60-245	61-406
DF + FA						
mean, µg/d	246	311	326	521	394	494
p5-p95, µg/d	144-396	175-483	152-573	284-841	211-608	248-810
200 µg FA/100 g bread						
FA only						
mean, µg/d	187	230	242	424	286	444
p5-p95, µg/d	82-348	86-435	71-471	180-780	120-490	122-812
DF + FA						
mean, µg/d	340	426	446	733	537	716
p5-p95, µg/d	191-555	29-691	196-803	690-387	273-847	322-1201

y = years of age
p5-p95 = percentiles 5 to 95
FA = Folic Acid
DF = Dietary Folate

At the highest mandatory fortification of bread (200 µg/100 g) 78% of the women of childbearing age would reach RI. The equivalent for pregnant women was 81%. In the same scenario the children aged 4 and 9 years had the highest intake, approximate 50-60% of them would get an intake of total folate over UL. At the lowest level of

fortification of flour (140 µg/100 g), 18%, 19% and 7% of children aged 4, 9 and 13 respective, would get an intake over UL (table 7).

The scenario where least persons get an intake over UL is when bread is fortified with 100 µg/100 g. This study show that only 19% of the children aged 4 years and 15% aged 9 years reaches UL. However, in this case 56% of the fertile women and 57% of pregnant women did not reach the recommended intake (table 7).

Table 7: Percentage of the Norwegian population, by age group, receiving folate intakes, below, according to and above the NNR recommendations, food folate and FA under simulated fortification scenarios of flour and bread.

DietaryFolate	Children 4 y	Children 9 y	Children 13 y	Men 16-79 y	Women 16-49 y	Women pregnant
+140 µg FA/100g flour						
% < RI	0	1	12	8	57	71
% RI	82	80	81	90	41	29
% > UL	18	19	7	2	0	0
+190 µg FA/100 g flour						
% <RI	0	1	8	4	42	58
% RI	66	65	80	92	57	41
% > UL	34	35	12	4	1	2
+240 µg FA/100 g flour						
% <RI	0	0	6	2	30	45
% RI	50	49	75	91	70	51
% >UL	50	51	19	7	0	4
+100 µg FA/100g bread						
% <RI	0	1	15	7	56	57
% RI	81	84	82	91	44	42
% >UL	19	15	4	2	0	1
+200 µg FA/100 g bread						
% <RI	0	1	6	1	22	19
% RI	41	46	77	85	76	68
% >UL	59	53	17	15	2	13

y = years of age
RI = Recommended Intake
UL = Upper Level

4.3 Voluntary fortification of some food

Estimated folic acid intakes of the groups consuming specific amounts of FA from voluntary fortified foods are presented in table 8. Milk is the product that contributes with most FA. If the groups choose voluntary fortified milk, it contributes with almost: 38, 26, 24, 21, 7 and 10% of the recommended daily intake for the groups respective.

For the pregnant women the average intake of folate would increases from 272 µg to 349 µg, if they choose all products. This means that 25% more of the pregnant women reaches the recommended intake. The corresponding number for fertile women is 10%.

Table 8: Folic acid intakes from voluntary fortification µg/day mean (p.5 p.95)

Product	Children 4 y	Children 9 y	Children 13 y	Men 16-79 y	Women 16-49 y	Women pregnant
Breakfast cereals mean, µg/d p5-p95, µg/d	 9 0-38	 12 0-64	 9 0-48	 10 0-47	 7 0-33	 8 0-40
Juice mean, µg/d p5-p95,µg/d	 2 0-12	 4 0-20	 5 0-24	 6 0-26	 6 0-27	 10 0-27
Nectar mean, µg/d p5-p95, µg/d	 1 0-10	 2 0-12	 1 0-11	 1 0-4	 1 0-4	 6 0-32
Yoghurt mean, µg/d p5-p95, µg/d	 9 0-26	 7 0-26	 6 0-28	 3 0-18	 5 0-20	 11 0-34
Milk mean, µg/d p5-p95, µg/d	 30 6-59	 34 3-72	 26 0-70	 43 0-42	 27 0-37	 43 4-111
All five products mean, µg/d p5-p95, µg/d	 52 16-104	 59 14-131	 48 5-116	 63 8-150	 47 7-112	 77 16-169

y = years of age
p5-p95 = percentiles 5 to 95

The 4 years old children are the group who get most FA from the voluntary fortified food per 10 MJ, presented in table 9. If they used all the products the voluntary fortification will provide 65% of the daily recommendation.

Table 9: Folic Acid intake from voluntary fortified: Breakfast cereals, Juice, Nectar, Yoghurt and Milk µg/ 10 MJ, mean (p.5 p.95)

Voluntary fortification	Children 4 y	Children 9 y	Children 13 y	Men 16-79 y	Women 16-49y	Women pregnant
FA concentration mean, µg/10 MJ p5-p95, µg/10 MJ	 83 27-159	 30 0-96	 55 7-123	 57 9-122	 56 10-118	 76 20-148

y = years of age
p5-p95 = percentiles 5 to 95

4.4 Voluntary fortification and supplements

Of the fertile women and pregnant women 83% would get an intake over RI, if taking FA supplement with 200 µg per day in addition to voluntary fortification and dietary folate.

The percentage of participants with risk of an intake above the UL for the groups consuming specified amounts of FA from voluntary fortified foods and dietary supplements (200 µg) in addition to dietary food folate, are presented in Table 10. None of the groups reached UL from just the fortification and supplements, but taken into account the contribution from dietary food folate. Almost all the children aged 4, and 75% aged 9 years, are risking to reach a daily intake of folate above UL if taking FA supplement with 200 µg per day in addition to voluntary fortification and dietary folate.

Table 10: Folate intakes from voluntary fortification selected foods and if they are taking a FA supplement with 200µg in addition to dietary food folate µg/day mean (p.5 p.95)

	Children 4yy n = 391	Children 9 y n = 810	Children 13 y n = 1005	Men 16-79 y n = 1291	Women 16-49 y n = 928	Women pregnant n = 19711
Intake of folate mean, µg/d p5-p95, µg/d % >UL	405 314-525 99	454 338-614 75	453 319-654 9	572 400-827 2	496 352-694 0	549 375-791 1

y = years of age
p5-p95 = percentiles 5 to 95
UL = Upper Level intake (see table 2 for recommended levels)

4.4.1 Total Folic Acid from all sources

The results from my model calculations including the assumption that flour or bread are fortified with FA to varying degrees, in addition to the voluntary fortification and use of a supplement of 200 µg, is presented in table 11. At the lowest level of mandatory fortification of wheat flour (140 µg/100 g), it was estimated that only 2% of the women of child-bearing age would get an folate intakes below 400 µg/day, and with the lowest mandatory fortification of bread (100 µg/100 g) 3% of the women would get an intake below 400 µg/day.

Children aged 4 years old were the only group to get an intake of total FA over UL in all scenarios. All children aged 9 years reached UL in the two highest levels of the flour fortification scenarios and the highest level of fortification of bread. None of the other groups reached an intake over the UL.

Table 11: Intake of Folic Acid µg/day from voluntary fortification and 200 µg FA-supplements and FA from different mandatory fortification scenarios, mean (p5 - p.95)

FA from voluntary fortification and 200µg FA supplements	Children 4 y n = 391	Children 9 y n = 810	Children 13 y n = 1005	Men 16-79 y n = 1291	Women 16-49 y n = 928	Women pregnant n = 19711
+140 µg FA/100 g flour						
mean, µg/d	344	387	383	461	384	441
p5-p95, µg/d	273-426	287-503	270-537	324-656	289-508	298-633
+190 µg FA/100 g flour						
mean, µg/d	377	433	431	532	433	500
p5-p95, µg/d	291-477	308-588	285-628	356-781	313-592	320-742
+240 µg FA/100 g flour						
mean, µg/d	410	479	479	603	482	559
p5-p95, µg/d	305-528	330-673	302-726	387-902	334-679	340-853
+100 µg FA/100 g bread						
mean, µg/d	345	374	369	784	390	499
P5-P95, µg/d	282-431	277-497	261-515	516-1152	285-526	319-718
+200 µg FA/100 g bread						
mean, µg/d	439	489	490	996	534	721
p5-p95, µg/d	328-599	328-702	298-743	617-1520	346-763	387-1115

y = years of age
p5-p95 = percentiles 5 to 95

5. Discussion

My results suggest that it is impossible to fortify food with FA in Norway at a level that ensures that the majority of women in the target group will consume 400 µg folate/day, without more than 5% in other population groups being exposed to folic acid in amounts exceeding the UL. This is accordance with a similar report from New Zealand (128).

Folate has received much attention as a vitamin that can protect against many diseases. According to the results from Norkost 88% of men reach the intake recommended in NNR, whereas the results from MoBa indicate that only 3% of the pregnant women reach the recommended intake from non fortified foods. Several strategies to increase the folate status in the population have been discussed in Norway (129), one of them is mandatory fortification. If mandatory fortification of flour with folic acid was introduced in Norway at the same level as it is in USA (140 µg FA/100 g flour), 41% of the fertile women and 29% of the pregnant women would reach the RI. In the same scenario almost 20% of the children aged 4 and 9 years would exceed UL. This is in accordance with a recent Swedish report (130). At the highest level of mandatory fortified wheat flour (240 µg/100 g) it was estimated that 30% of fertile women and 45% of the pregnant women still would receive folate intakes below RI. The highest folate intake with this fortification strategy would be among children aged 4 and 9 years, more than 50% of them would have an intake of total folate over 300 µg and 400 µg respectively.

Mandatory fortification is non-specific and it affects both the target group and all non-target groups. For this reason, fortification must be safe for all groups. In this study the most vulnerable group is the children aged 4 years. This is in agreement with other studies (68;114). In my study more than half of them would get more than UL of total folate, if the flour had been enriched with 240 µg FA. None of them would reach UL of FA only. When adding voluntary fortification and supplement almost all of them get more than 300 µg FA.

The hypothetical intake in this study does only represent the part of the population that use the voluntary fortified products. In the scenarios with supplement, 200 µg have been added to all groups even for children aged 4 years, this might be a to high estimation. According to Ungkost only 42% of the 4 years old children who used supplements containing FA, had an intake of 200 µg FA from supplements. On the Norwegian market to day there are a lot of different supplements designed for children, most of them contain 75-200 µg FA.

If Norway as the US should fortify flour with 140 µg/100 g in addition to voluntary fortification and a supplement with 200 µg FA, the average intake of FA would be 344, 387, 383, 461, 384, 441µg, for 4, 9, 13 years old children, men, fertile women and pregnant women respective. This will come in addition to the dietary folate. This scenario will not be realistic in Norway, as long we have the law we have today, because if Norway go for mandatory fortification, voluntary fortification with FA will not be permitted.

The folic acid intakes estimated in this study may be overestimated. In the hypothetic mandatory fortification scenarios all wheat flour and all bread have been fortified. The last year the import of "bake of" and dough has increased in Norway, approximate 10% of bread is imported today (131). On the other hand, we do not know whether these products might be fortified in the future.

5.1 Choice of potential food vehicle for mandatory fortification

Wheat flour fortification is a good choice, because there are very few flour mills supplying the Norwegian market. Thus, the task of implementing and monitoring FA fortification of flour would be simpler than for foods with numerous manufacturers and suppliers.

Breads fortified at 200 µg/100 g provided folate to the greatest number of the target groups, 80% of them reached RI. In Norway there are five main bread producers. The

largest of them provide approximate 30% of the bread in Norway therefore, as for wheat flour, the task of implementing and monitoring fortification should be relatively simple. Fortification of bread would result in significantly fewer foods being fortified than if flour were to be fortified, thus providing more choice for consumers.

Celiac patients should also be considered since they have a higher total plasma homocysteine level than the general population, indicative of a poor vitamin status (132). Their diet would not be reached, if we just choose wheat flour or traditional bread, and they might be vulnerable to inadequate intake of folate. If it will be mandatory fortification in Norway their diet should be considered separately and gluten free flour would have to be fortified in the same amount as regular flour.

Liquid milk can be a potential food vehicle for mandatory fortification. Studies have suggested that folic acid fortified milk is an efficacious method increasing folate status (133). In this study I did not include milk as a potential product for mandatory fortification because children are frequent consumers of milk, and so may be exposed to excessive amounts of folic acid. This is a decision I regret, especially because it seems to be a good source for pregnant women. In addition, as for wheat flour, the task of implementation and monitoring of fortification should be relatively simple because there are only two main dairies in the Norwegian market.

5.2 Recommendations

In the last decade the folate recommendations from NNR has increased. For women in fertile age the recommendation before 1996 was 200 µg, in NNR -96 it increased to 300 µg, the last recommendation in NNR -04 is 400 µg total folate. The value for RI includes a margin accounting for variations in requirement and physiological factors influencing the requirement. It is important to distinguish between the average requirement (AR) for folate and the recommended intake. The recommended intake represents more than the requirement for the average person and also covers the

individual variations in the requirement for the vast majority of the population group. In this study I have only used RI because it is important that as many of the target group as possible get enough folate to reduce the risk for NTDs. If using AR instead of RI 76% of the fertile women would reach the recommendation from non-fortified food and that indicate there are not so many individuals who need an increased folate intake. In addition to that, all women in childbearing age are not capable of becoming pregnant, and not all women capable of becoming pregnant will be so. In that point of view the amount of women who need 400 µg folate decreases.

In Norkost the FFQ is the method used in assessing food intake. The ability for this method to quantify the absolute intake of foods and nutrients is in general limited. Even if we had known the absolute intake, it must be emphasised that the intake value can never decide whether the intake is adequate or not, it can only indicate the probability that it is adequate. Knowledge of folate status in Norway is limited. Although a few studies have measured folate in blood, this has not been related to disease, and there is little knowledge of the distribution of potential folate deficiency in subgroups of the population (134). The calculated folate intake in this study is not absolutely true values because the food composition tables which have been used have an error margin. There is today no analytic method with a status as reference method for the measurement of natural folate in foods since all methods are complicated by difficulties in sample preparation, including extraction, deconjugation and purification (131).

The term UL can be very confusing. The “tolerable-upper-limit concept” was used by the US Food and Drug Administration (FDA) to limit the folic acid concentrations required in fortification. NNR-96 also based its recommendation on total folate consumption, with an upper limit of 1000 µg a day. Later it has been noted that it was incorrect to set a tolerable upper limit of total folate, rather it should be set in terms of synthetic folic acid only, and so it is in NNR -04. There is no evidence for risk associated with high intake of folate from natural sources and there are different views about the risk related to more than 1000 µg FA (13). The UL for children and

adolescents is adjusted on the basis of mean bodyweight. This is very uncertain level, it is possible that it can be much higher but it can be also much lower –we don't know before more studies have been done. Several intervention studies with FA supplementation are in progress to establish its effect on mortality and morbidity (47), unfortunately none of them are designed to discover any side effects of a decreased use of FA.

The FA will always be in addition to folate from food. So far, we don't know if high intake of folic acid in addition to dietary folate can be a risk for children. More studies are needed. It may be that a complex intake of DF and FA needs to be studied over long time, before conclusions are made regarding UL. Because of this I have estimated both the total folate intake and the intake of FA. This is in agreement with the advice from The Swedish Council on Technology Assessment in Health Care (SBU) to The Swedish National Administration (SLV) (130).

The mix of dietary folate and folic acid can be confusing because the bioavailability of folate is 50-98% relative to FA depending on the folate source (13-15). Although the differentiation between folate forms is important, I have not accounted for the differences in bioavailability of natural folate and FA in this study, because in the NNR the bioavailability of the food folate have been taken into consideration. If NNR as US had used DFE, a higher amount of people in my hypothetical models of fortification, would have reached an intake according to RI.

5.3 Vitamin B12

There have been no reports of epidemics of neurological complications due to vitamin B12 deficiency, in countries that have mandatory fortification (68;77). With the possible consequences of masked vitamin B12 deficiency from folate fortification (29), perhaps it would be better to fortify with both folic acid and vitamin B12. Concentrations of B vitamins are frequently inadequate in late life, folate as well, and many of the elderly will get benefits from fortification. Fortification with vitamin

B12 would not only help elderly, it will also benefit vegetarians and alcoholics who are known to have risk for low vitamin B12 status.

There are also other risks than “masking” B12 deficiency. Recently a report from India show that children born of mothers with low B12 but high folate status were the most adipose and the most insulin resistant (135). The imbalance between folate and vitamin B12 are also associated with other adverse effects as a relation between high serum folate and cognitive impairment is reversed in subjects who have a low vitamin B12 status(136). The problem is that we do not know if it can be any unexpected outcomes. There can be a risk that high folate and high vitamin B12 status together can influence other metabolic effects.

5.4 Strategies

Supplementation with folic acid and stimulation of the consumption of foods naturally rich in folate are both useful strategies to increase folate status (33). However, these two strategies are not effective at a population level for the prevention of NTD. In Norway it is suggested that approximately half of the pregnancies are unplanned (33). Then the recommendation that supplementation should start before conception is simply not realistic.

As soon as the outcomes of the clinical trials of NTD became known and accepted, public health authorities in most countries, Norway included, advised women of childbearing age to increase their daily intake of folate to 400 μg. Although the advice was sensible and prudent, now more than a decade later, it is clear that putting an effective prevention strategy based on public awareness into place has been much more difficult than originally anticipated. Unfortunately, FA-promotion efforts targeting childbearing women haven’t been as successful as expected in decreasing the level of NTD. However, there has been an increased knowledge of folate in the target group (137). And the use of diet supplements has expanded over the last decade (137). Despite the measures taken to date, the majority of women in all

countries surveyed are not taking FA supplements periconceptionally. The big challenge is the timing. Mandatory fortification affects the whole population and covers also unplanned pregnancies, and this appears to be the most effective public health strategy to prevent NTD, even though stimulation of folate rich food and promoting the use of FA supplement should be used at the same time.

Only five products were used in the modelling of voluntary fortification in this study. According to the results the FA intake was increased with 52, 59, 48, 63, 47 and 77 µg respective for 4, 9, and 13 years old, men, fertile women and pregnant women. The minimum amount of FA required to prevent NTD will never be known with certainty because further randomised trials are unethical. Therefore, any strategy that increases red blood cell folate in women of childbearing age should reduce the number of NTD-affected births. There are several reasons to believe that it will be a higher intake of folic acid from voluntary fortification in the future. First it is possible that more products will be enriched. According to German estimates it can be realistic to account that up to 20% of the total diet can be voluntary enriched in the future (138).

Another reason is that manufacturers may also add more folic acid than claimed on the food label. The use of overages when adding nutrients to food products is common practice in the food industry. According to reports from US, the folate content of a food may be considerably higher, in some cases over 200% higher, than that indicated on the food label (79;139).

A third reason is that the industry has the necessary resources to inform the population through campaigns. Commercial companies have a larger ability to give a message to the people than a governmental founded campaign. If the commercial companies see a possibility to make money on folic acid's health benefits, they are probably willing to invest a rather large amount of money to be able to release the first product on the market.

If you on top of this add that FA supplements uses are increasing, the room for mandatory fortification decrease. In this study, fortification scenarios for those taking supplements with 200 μg FA in addition to the five voluntary products and dietary folate show that 99% of children aged 4 years and 75% aged 13 years received total folate over UL. The fact that some of the supplements designed for children contain 200 μg FA can be questioned, especial if Norway goes for mandatory fortification but also with regards to voluntary fortification. It might be a good decision to limit the content of FA in supplements designed for children.

5.5 The complex mixture of micronutrients

Low consumption of fruits and vegetables has been consistently related to an increased risk of a number of diseases (14;52;140;141). Many components of fruits and vegetables may be responsible for the reduced risk. Evidence that a high intake of vegetable and fruits reduces health-risk has led to attempts to isolate specific nutrients and administer them as supplements, sometimes in very high doses. Most of these attempts have been unsuccessful in reducing the precursors or preventing diseases and even in some cases have had adverse effects (142;143). The complex mixture of micronutrients found in a diet high in fruit and vegetables may be more effective than large supplemental doses of a small number of specific micronutrients. It could be that several of these compounds work together, but have no effect individually, or that other dietary components may be effectors of micronutrient action (144).

Randomized intervention trials of micronutrient supplements have, to date, largely failed to show an improvement in clinical end points. Studies concentrating on whole foods or diet pattern may be more effective in demonstrating an effect on clinical end points. As mentioned earlier, Larsson et al. reported an inverse association between intake of folate from foods and the risk of cancer (55). But they didn't see the same association between supplemental folic acid intake and cancer risk, this have been confirmed in other studies (56;57) and by a recent meta-analysis (52). So to reduce the incidence of diseases like cancer and CHD, the best advice presently is to

consume nutrients through food sources rather than supplements. But it is not realistic, at the present time, to advice women to meet the additional 400 µg per day target by increasing their intake of folate rich foods, because it will take a long time to make such a change, and it may be difficult for all women to achieve a sufficient intake

Kim and colleagues have shown that the dose and timing of folate interventions may be critical. Modest doses of FA supplementation suppressed the development and progression of colorectal cancer (80;145). These studies show that the interventions are critical in providing safe and effective chemoprevention. In this regard, it is noteworthy that folate antagonists are used in cancer treatments. It is, therefore, theoretically possible that high intakes of supplemental FA, which is more bioavailable than folate from foods, may promote the progression of cancer in individuals with already existing, undiagnosed cancer (55).

It is now beyond doubt that folate has a key role in homocysteine metabolism, and that high plasma Hcy concentrations can be lowered by FA supplementation or food folates. In the NORVIT study there was a reduction in Hcy in those treated with folate and B12, but no effect on myocardial events was seen (48). In the HOPE-2 study, despite a reduction in Hcy, again vitamin treatment had no effect on myocardial events (146). It seems therefore clear that FA does not reduce the risk of atherosclerosis by reducing Hcy, even if folate intake is an important predictor of plasma Hcy. This is a parallel to The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study in 1994. By the early 1990s, there was mounting clinical epidemiologic evidence of reduced cancer risk associated with increased intake of antioxidants. Treatment with vitamin E and beta-carotene were considered unlikely to be harmful and likely to be helpful, and the question was asked whether antioxidants could reduce lung cancer in high risk groups like smokers. No benefit was seen with either therapy, but compared to placebo a disturbing worsening trend was seen in the beta-carotene-treated group (142). We have to see in a larger perspective, and sort out the right marker. Folate is not the only factor that correlates with elevated Hcy

concentrations, it can also be the result of low intakes of vitamin B12, poor kidney function, smoking, coffee consumption, high body weight or mutations in the folate-metabolizing enzymes (147). A high tHcy concentration may just be a marker of low vitamin status, or an unhealthy lifestyle rather than a true causal risk factor. The relation between intermediates in the Hcy pathway and health-risk is therefore complex and requires many further studies.

A common variant of the MTHFR gene has been identified with a change of a cytosine to a thymine at nucleotide 677, and this variant is associated with reduced enzyme activity. The TT genotype is associated with an increased risk of having a child with a neural tube defect (148) and is a risk factor for some types of cancers (52). The modulation in human health risk between MTHFR genotypes can be accentuated by low folate status (149;150). The MTHFR 677TT genotype also affect the sensitivity of patients to folate based chemotherapeutic cancer treatments (151).

The foetus is not only risking NTDs. The MTHFR TT genotype of the mother has been shown to increase the risk of Down's syndrome especially if the folate status in the preconception period is low (150).

Genetic variation certainly has an important influence on human nutritional requirements. New knowledge of the complexity of the interaction between genes and diet offered opportunities to recommended optimal nutrition for individuals in the future. In the meantime it is important to be humble and have courage to admit there is much we don't know yet.

5.6 Unanswered questions

We need answers to what will happen if we go for mandatory fortification -before we start a program.

We have to know more about the folate status in the population

A number of questions regarding the bioavailability of natural and added folates and the level of folate intake from dietary sources needed to meet nutritional requirements remain unanswered. Much more research is also needed to characterise better markers of micronutrient status both in terms of metabolic effects and health effects, so that risk persons can be identified and supplementation modified accordingly. It might be that a complex lifelong behaviour pattern needs to be studied before conclusions can be made regarding folate and disease.

How many will exeed UL?

In this study the children aged 4 years old, was the most vulnerable group. Even though it is not allowed to fortify food for small children in Norway, they will nevertheless get a higher intake of FA if mandatory fortification is implemented. After FA fortification started in US it is the children less than 5 years who have the highest serum folate concentration in the US population (68).

I did not consider children less than 4 years in my dietary modelling. This is because The Norwegian Food Safety Authority defined them as an own category, and don't include them in the model of fortification. The UL for children 1–3 years is 200 µg/day, which is much lower than that for adults, the risk of exceeding the upper limit in this group may be greater. In *Spedkost and småbarnskost*, nationwide surveys of Norwegian children aged 6, 12 and 24 month, it has been reported that the two years old children (n=1720) had an intake of 95g bread/day, witch means they are risking a high intake of FA in case of fortification. In the US, Lewis et al estimated that 15–25% of children aged one to eight years had FA intakes exceeding their UL under the current US fortification program (114).

Although the women capable of becoming pregnant are the target group, some of them are also risking an intake over UL. Information from the database of the Spanish Collaborative Study of Congenital Malformations (152), reveals that, in 2004, more than 60% of women of healthy children who had been using FA

supplement since before the present pregnancy had ingested higher doses than recommended. The proportion using more than 1 mg was 63%. The same results were seen in women who started to take FA supplements after becoming pregnant (152). This is perhaps one of the biggest problems, it is common that the people who eat most supplements also are the one who eat and live most healthy. If they in addition have opportunity to choose voluntary fortified products it is not unrealistic to suppose they will choose them too.

If the pregnant women use too much FA it can also affect the foetus. Recently a study from Ireland of 11 infants showed that all exhibited unmetabolised FA in their cord blood. None of the mothers were consuming FA supplements. The levels of unmetabolised folic acid detected in the cord blood must therefore be due to recent dietary intake of FA from commercially available fortified foods on the Irish market (153). It is unknown if the presence of FA in cord blood will imply any risk, but the likelihood is that the longer the exposure the more likely the potential for harm.

Monitoring

Monitoring is very important, not only both of the industries production and labelling but also of the folat/folic acid status in the population.

We need clear instructions before it is possible to start a mandatory fortification. It is important that the product don't contain more than it has been permitted to contain. If Norway goes for mandatory fortification, voluntary fortification with FA will not be permitted, then we also need monitoring to ensure that import products have not been fortified of mistake, because most of the states Norway imports from have voluntary fortification. Most of all we need a monitoring plan to identify adverse health effects in time.

The labelling issue needs also to be considered. If the wheat flour will be fortified, products like cakes, hamburger bead and pizza will also be fortified. What happen if new EU regulations forbid a claim for fortification to be made, for example if the product is deemed to be too high in fat, salt or sugar? EU will probably forbid health

claims on that type of foods, but fortification in all foods made from fortified flour will have to be declared.

6. Conclusion

In NNR 2004 the UL for folate is only valid for FA not dietary folate. There is a very small risk to get a FA intake above the UL. If the FA intake is in addition to the dietary folate the risk is much higher. The most vulnerable group would be the youngest children.

It is clear that the relationships between folate and health outcomes are complex; further, we need a better understanding of the relevant biological mechanisms to avoid misinterpretation. And we need to know more about the effects of chronic exposure to FA before instituting mandatory fortification. It is important that medical doctors and other health care providers continue to encourage women of child-bearing age to increase folate intakes from foods and dietary supplements whether mandatory fortification proceeds or not.

In the late 1990`s when US started the mandatory fortification, the optimism was high that FA didn't just promote NTD but had a lot of benefits for the whole population. To day it is more uncertainty, the only thing we know for certain is that FA promote 50-70%of the NTDs. In Norway it will say at best less than 50 cases a year.

If it is unlikely that any fortification level would prevent all of the preventable NTD, is it then ethical to expose the whole population without choice, for FA? We need to ask the right questions and undertake appropriate research. It is quite likely that there will be no adverse effects from chronic, even a lifetime exposure to FA, but we do not know this for certain. For that reason, we have to wait with mandatory fortification.

Each day scientists discover new genes sequences. As the interactions between genetic variation and nutritional requirements become more studied, it will allow dietary recommendations to be individualised according to genotype to ultimately reduce our risk of a lot of diseases and increase health. Meanwhile, we can

recommend consuming a well-balanced diet with a variety of foodstuffs, be physically active, maintain a normal body weight, avoid smoking and drink less alcohol. This will benefit the whole population.

Most of all we need to be humble and always have in mind that we do not have all the answers about the mystery of life.

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Appendix 1

Calculation

JUICE

- 100 g Juice 43kcal
- 100kcal (100/43 =2,3259g/kcal) 2,3259*100=232,59g/100kcal
- 100kcal 20mcg (20 / 232,59 =0,086 mcg/g)
- 8,6 mcg /100 g juice
- 9 mcg

NEKCTAR

- 100g 42kcal
- 100/42 *100 = 238 g/100kal
- 20/238 = 0,084 mcg/g
- 8,4 mcg/100g
- 8 mcg

YOGHURT

- 100g natural: 79kcal
- 100kcal ((100/79)*100)= 126,58g
- 20mcg/100kcal = 20/126,58g (=0,158 mcg/g)
- 15,8mcg/100g
- 16 mcg

BREAKFAST CERIALS UNSWEETED

- 100 g 345 kcal
- 100/345 *100 = 28,99g

- 20 mcg / 100kca = 20mcg / 28,99g (= 0,69 mcg/g)
- 69 mcg / 100g

BREAKFAST CERIALS SWEETED

- 100 g 385kcal
- 100/385 * 100 = 25,97g
- 20 mcg/ 100kcal = 20mcg/25,97 g (0,77)
- 77 mcg / 100g

H-MILK

- 100g 66kcal
- 100/66*100=151,515
- 20 mcg/100 kcal = 20/151,515g (0,132)
- 13 mcg / 100g

MILK 1,5% Fatt

- 100g 46 kcal
- 100/46*100=217,391
- 20mcg/100 kcal=20/217,391 (0,092)
- 9 mcg/100 g

MILK 0,7 % fatt

- 100g 38 kcal
- 100/38*100=263,158
- 20mcg/100 kcal=20/263,158 (0,076)
- 8 mcg/100g

MILK 0,1% Fatt

- 100g 33 kcal
- 100/33*100=303,03
- 20mcg/100kcal=20/303,03 (0,066)
- 7mcg/100g

Appendix 2

The Norwegian Mother and Child Cohort Study (MoBa)

Pregnant women are recruited to the study through a postal invitation after they have signed up for the routine ultrasound examination in their local hospital. At the ultrasound examination, participating women and their partners donate biological samples. After birth, a blood sample from the umbilical vein is collected and a second blood sample is taken from the mother. Participants are asked to provide biological samples and to answer questionnaires covering a wide range of information up to age 7 for the child. The cohort is linked to national health registries to include certain outcomes. The study has been approved by the regional committee for ethics in medical research and the Data Inspectorate. The sample used here includes 19.711 pregnancies using version 1 of the quality-assured data files made available for research in 2005.